REMARKS

Claims 1-50 are pending. Claims 4-9, 13-17, 23-28, 45-46, and 49-50 are rejected. Claims 1-3, 10-12, 18-22, 29-44, and 47-48 have been cancelled in this Amendment. Therefore, Claims 4-9, 13-17, 23-28, 45-46, and 49-50 now remain pending.

This amendment is to accompany a Request for Continued Examination and is for the purpose of putting the claims in proper form for allowance. Claims 1-3, 10-12, 18-22, 29-44, and 47-48 have been cancelled and Claims 4 and 23 have been amended to limit them to subject matter enabled by the specification. Therefore, no new search is believed to be required by the amendments.

Claims 1-3, 10-12, 18-22, 29-44, and 47-48 have been cancelled pursuant to a restriction requirement in which the applicants had elected without traverse to prosecute Claims 4-9, 13-17, 23-28, 45-46, and 49-50 (Paper No. 4).

Claim 4 has been amended to recite that the vaccine comprises a recombinant polypeptide that consists essentially of at least one epitope from the 16 (±4) and at least one epitope from the 30 (±4) kDa antigen. Support for this amendment can be found in the specification in the paragraph bridging pages 15-16, page 16, lines 22-24, and the sentence bridging pages

16-17.

Claim 23 has been amended to recite that the method is an *in vitro* method for producing a fusion polypeptide that consists essentially of at least one epitope from the 16 (±4) kDa antigen and at least one epitope from the 30 (±4) kDa antigen linked to a polypeptide that facilitates isolation of the fusion polypeptide. Support for this amendment can be found throughout the specification. For example, support can be found in the sentence spanning pages 16-17, and on page 18, lines 8-14; page 19, lines 7-8; page 19, lines 18-21; page 20, lines 6-9; and, page 20, lines 24-28.

The paragraph beginning on page 13, line 1, has been amended to reflect that U.S. Serial No. 09/156,954 relating to an antigen test to detect the 16 kDa and 30 kDa antigens of *Sarcocystis neurona* issued as U.S. Patent 6,153,394 on November 28, 2000.

Enclosed is a copy of the International Search Report for the applicants' International Application, PCT/US00/24221, and a copy of the reference it cites. The Search Report identified only Liang *et al.* (Infect. Immun. 66: 1834-1838 (1998)) as particularly relevant. Liang has been cited by the Examiner in Paper Nos. 3 and 5.

1. Claims 4-9, 13-17, 45-46, and 49-50 were rejected

under 35 U.S.C. § 112, first paragraph.

The rejection states that the claims to the vaccine are not enabled because the specification is prophetic and does not teach whether the vaccine would work, particularly in light of the disclosures of Liang *et al.* and Kisthardt *et al.*

The applicants disclose a vaccine that contains a recombinant polypeptide that comprises one or more epitopes from the 16 (±4) kDa and 30 (±4) kDa antigens. Kisthardt teaches that as of February 1997, a vaccine against *Sarcocystis neurona* is not available. Liang teaches that antisera from *Sarcocystis neurona* infected horses contain antibodies against 14 kDa and 16 kDa antigens which are neutralizing whereas the antisera contain antibodies against a 30 (±4) kDa antigen which are not. Liang had shown that result by neutralization tests which measured shizonts that appeared after incubating merozoites with the antisera. The results showed that antibodies in the antisera against the 16 kDa antigen caused the number of shizonts to decrease whereas antibodies against the 30 (±4) kDa antigen did not. While the results indicated that antibodies against the 30 (±4) kDa antigen were not neutralizing *in vitro*, the results did not indicate what effect antibodies against the 30 (±4) kDa antigen would have *in vivo*. The antibodies may have a role in preventing the

Sarcocystis neurona from invading neural tissue.

The primary object of the applicants' vaccine is to inhibit *Sarcocystis neurona* from invading neural tissue, not necessarily to kill the *Sarcocystis neurona;* therefore, the applicants' vaccine is not necessarily dependent on neutralizing antibodies. All the vaccine requires is that the antibodies interfere with the ability of *Sarcocystis neurona* to invade neural tissue.

Liang states on page 1834, "The high rate of exposure . . . and the relatively low incidence of clinical EPM indicate that most horses develop effective immunity that may prevent entry into the central nervous system . . . ." Consistent with that statement, Liang and the applicants show that horses have antibodies against several *Sarcocystis neurona* antigens, in particular the 16 (±4) and 30 (±4) kDa antigens. Therefore, it is plausible that antibodies against one or both of these antigens are involved in preventing *Sarcocystis neurona* from invading neural tissue.

The applicants state that the 30 (±4) kDa antigen is specific to *Sarcocystis neurona* whereas Liang states that 30 (±4) kDa antigen is not; however, whether antibodies against the 30 (±4) kDa antigen identified by Liang are specific to only the 30 (±4) kDa antigen of *Sarcocystis neurona* is not relevant to the applicants' vaccine, which is to prevent *Sarcocystis neurona* from

invading neural tissue. Even an antibody that cross-reacts with several species can still be effective against *Sarcocystis neurona*.

While the horses that were sampled in Liang had all been diagnosed as having a neurological disorder resembling EPM, it does not necessarily follow that because antisera from the horses contained antibodies against the 16 (±4) and 30 (±4) kDa antigens, a vaccine comprising the 16 (±4) and 30 (±4) kDa antigens would be ineffective in preventing spread of *Sarcocystis neurona* into neurological tissue.

Historically, many vaccines have been developed against a variety of pathogens, which contain antigens that had first been identified by reactivity with antisera from individuals displaying the disease caused by the pathogen. Vaccine development has operated under the following premise. The antisera from these diseased individuals allowed the antigens from the pathogen that stimulated the individual's immune system to be identified and it is these antigens which provided in a vaccine would induce an immune response that would protect an individual against exposure to the pathogen. Therefore, the purpose of a vaccine is to boost antibody titers in the individual so that when the individual is exposed to the pathogen addressed by the vaccine, the immune response is more effective than it otherwise

would be. Therefore, even though a diseased individual may have antibodies against a particular antigen, it does not necessarily follow that a vaccine containing that antigen would not be effective.

Liang (page 1836) teaches that the 16 (±4) kDa and 30 (±4) kDa antigens are surface antigens. Because surface antigens are generally important in the function or life-cycle of the organism, it is reasonable to presume that blocking the activity of the antigens by binding with antibodies would interrupt the function or life-cycle of the *Sarcocystis neurona*. Therefore, a vaccine that contains the 16 (±4) and 30 (±4) kDa antigens would enable a vaccinated horse to have antibody titers that are sufficiently high to bind all of the *Sarcocystis neurona* in the horse and thereby prevent it from entering the horse's neural tissue whereas in a non-vaccinated horse, by the time the horse has produced sufficient antibody titers against the *Sarcocystis neurona*, it has already entered the horse's neural tissue.

Therefore, in view of the knowledge charged to one with ordinary skill in the art of vaccine development, the applicants' disclosure relating to a *Sarcocystis neurona* vaccine comprising a recombinant polypeptide comprising one or more epitopes from the 16 (±4) kDa and 30 (±4) kDa antigens is enabling.

Reconsideration of the rejection is requested.

2. Claims 4-9 and 23-28 were rejected under 35 U.S.C. § 112, second paragraph.

(a) The rejection states that Claims 4-9 were rejected because in Claim 4 it is unclear what the term "recombinant" modifies and what combinations the phrase "combinations thereof" refers to.

Claim 4 has been amended to recite that the vaccine comprises a recombinant polypeptide that consists essentially of at least one epitope from each of the *Sarcocystis neurona* antigens identified by the applicants. The amendment makes it clear that the vaccine comprises a recombinant polypeptide which has one or more epitopes from the 16 (±4) kDa and 30 (±4) kDa antigens. A recombinant polypeptide includes fusion polypeptides which are extensively discussed in the specification and is the preferred polypeptide for the vaccine. Therefore, regardless of whether the recombinant polypeptide contains one or more of epitopes from the 16 (±4) and 30 (±4) kDa antigens, the recombinant polypeptide is not the same as the naturally occurring 16 (±4) kDa or 30 (±4) kDa antigen. Reconsideration of the rejection is requested.

(b) The rejection states that Claims 23-28

were rejected because in Claim 23 it is unclear what the phrase "combinations thereof" refers to and it is unclear how "an additional polypeptide" is related to the fusion polypeptide.

Claim 23 has been amended to recite that the method is for producing a fusion polypeptide that consists essentially of at least one epitope from each of the *Sarcocystis neurona* antigens identified by the applicants linked to a polypeptide that facilitates isolation of the fusion polypeptide. The amended claim makes clear that the antigens and polypeptide are fused together into a single molecule. Therefore, the claim can no longer be read as describing two separate polypeptides. Reconsideration of the rejection is requested.

3. Claim 4 was rejected under 35 U.S.C. § 102(b) as being anticipated by Liang *et al.* because Liang teaches a 16 kDa protein that can be used in a vaccine.

The rejection states that because Claim 4 can be read as relating to a vaccine that contains only all of the epitopes of the 16 kDa antigen, the claim embraces a composition containing the 16 kDa protein of Liang.

Claim 4 has been amended to recite a recombinant polypeptide consisting essentially of at

least one epitope from each of the 16 (±4) and 30 (±4) kDa antigens. While Liang discloses an isolated 16 kDa protein and suggests that it may useful as a component in a vaccine to protect equids, Liang does not disclose nor suggest a vaccine comprising a recombinant polypeptide comprising at least one epitope from the 16 (±4) kDa antigen, particularly when it is linked to at least one epitope from the 30 (±4) kDa antigen, which is then fused to a polypeptide that facilitates isolation of the polypeptide. As the specification illustrates, the recombinant polypeptide in one embodiment is a fusion polypeptide wherein the amino or carboxy terminus of the antigen is fused with another polypeptide (paragraph spanning pages 15-16), which further includes fusion polypeptides that comprise only one or more of the epitopes of the antigens (paragraph spanning pages 16-17). Even in embodiments where the recombinant or fusion polypeptide includes the entire amino acid sequence of the 16 (±4) kDa antigen, the recombinant or fusion polypeptide is not the same as the naturally occurring 16 kDa antigen of Liang. Therefore, Liang does not anticipate the vaccine of Claim 4. Reconsideration of the rejection is requested.

4. Claims 23-25 were rejected under 35 U.S.C. § 102(b) 23-25 as being anticipated by Liang *et al*. because Liang

teaches a method isolating a 30 kDa protein from an infected equid.

The rejection states that because Claim 23 can be read as relating to a polypeptide containing only all of the epitopes of the 30 (±4) kDa antigen and does not limit the culturing method for producing the 30 (±4) kDa polypeptide, it embraces both *in vitro* and *in vivo* culturing method and, therefore, the *in vivo* method used by Liang to isolate the 30 kDa protein is embraced by Claims 23-25.

Claim 23 has been amended to recite that the method is an *in vitro* method for producing a fusion polypeptide that contains at least one epitope from each of the 16 (±4) and 30 (±4) kDa antigens linked to a polypeptide that facilitates isolation of the fusion polypeptide. Therefore, Claim 23 as amended is not anticipated by Liang which recites an *in vivo* method for isolating the naturally occurring 30 kDa protein. Reconsideration of the rejection is requested.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attachment is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE**."

In view of the above, it is believed that Claims 4-9, 13-17, 23-28, 45-46, and 49-50 are in proper form for allowance. This amendment is to put the claims

in proper form for allowance or in proper form for appeal. Notice of allowance or entry of this amendment is requested.

Respectfully,

Ian C. McLeod
Registration No. 20,931

MCLEOD & MOYNE, P.C.
2190 Commons Parkway
Okemos, MI 48864
(517) 347-4100
FAX (517) 347-4103

Encls: International Search Report for PCT/US00/24221
Liang *et al.* Infect. Immun. 66: 1834 (1998)

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at line 1 of page 13 has been amended as follows:

The present invention provides a vaccine that protects equids against *Sarcocystis neurona*. In a preferred embodiment, the vaccine consists of a 16 (±4) kDa antigen and/or 30 (±4) kDa antigen in a subunit vaccine. Preferably, the 16 (±4) kDa antigen and/or 30 (±4) kDa antigen are produced in a recombinant bacterium or ~~eukaryote expression vector~~ which produces the proteins which are then isolated to make the vaccine. In another embodiment of the vaccine, the vaccine is a DNA vaccine that comprises a recombinant DNA molecule, preferably in a plasmid, that comprises DNA encoding all or part of the 16 (±4) kDa antigen and/or 30 (±4) kDa antigen. In another embodiment of the vaccine, the recombinant DNA is inserted into a virus vector to provide a live vaccine which is a recombinant DNA virus. In U.S. [Serial No. 09/156,954, filed on September 18, 1998] Patent 6,153,394 to Mansfield, which is hereby incorporated herein by reference, it was disclosed that *Sarcocystis neurona* possesses two unique antigens, a 16

(±4) antigen and a 30 (±4) kDa antigen. These antigens do not react with antibodies from other *Sarcocystis* spp. Thus, these antigens are useful for producing vaccines that protect equids against *Sarcocystis neurona*.

In the Claims:

Claims 1-3, 10-12, 18-22, 29-44, and 47-48 have been cancelled.

Claims 4 and 23 have been amended as follows.

-4- (Twice amended)

A vaccine for active immunization of an equid against a *Sarcocystis neurona* infection comprising a recombinant polypeptide consisting essentially of at least one epitope of a [unique] 16 (±4) [or] kDa *Sarcocystis neurona* antigen and at least one epitope of a 30 (±4) [recombinant] kDa *Sarcocystis neurona* antigen [of *Sarcocystis neurona* and combinations thereof].

A method for producing a fusion polypeptide *in vitro* comprising:

(a) providing a microorganism in a culture containing a DNA encoding [a] the fusion polypeptide [comprising] consisting essentially of at least one epitope of a 16 (±4) kDa *Sarcocystis neurona* antigen [or] and at least one epitope of a 30 (±4) kDa *Sarcocystis neurona* antigen [or combinations thereof of *Sarcocystis neurona* and an additional] linked to a polypeptide that facilitates isolation of the fusion polypeptide;

(b) culturing the microorganism in a culture to produce the fusion polypeptide; and

(c) isolating the fusion polypeptide from the *in vitro* culture.